WATER RESOURCES RESEARCH GRANT PROPOSAL

Project ID: NY1841

Title: Epidemiologic Risk Analysis of Cryptosporidium parvum in Watershed: the Role of Genetic Variation Among Isolates

Focus Categories: Ecology, None

Keywords: Dairy Farms, Pathogens, Molecular Biology, Risk Assessment, Cryptosporidium parvum

Start Date: 03/01/2001

End Date: 02/28/2002

Federal Funds: $0

Non-Federal Matching Funds: $29,945

Congressional District: 26

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Abstract

Problem: Commonly used conventional and coproscopical diagnostic methods for C. parvum in fecal samples depend on morphological characteristics and have higher limits of detections, at least 500 oocysts per gram. Although the Cryptosporidium oocysts are morphologically identical there is considerable evidence of genetic heterogeneity among isolates which coincides with the host species, suggesting that some form of host adaptation has taken place, ie, not all isolates are infective to man. At present, eight valid species of Cryptosporidium are accepted (1,2). By being able to accurately identify the source(s) we can refine our risk assessment approach and implement cost-effective risk management strategies.

Objectives: The long-term objective is to develop an accurate risk model for C. parvum in watersheds so that cost-effect strategies can be devised and implemented to manage and eliminate its risk. The proposed studies represent a step towards that objective. The primary objective of the proposed studies is to expand our panel of polymorphic markers for the identification of a wider range of genotypes of Cryptosporidium spp. isolates. We are hypothesizing that Cryptosporidium isolates infecting different animal hosts have different genotypes and this can be exploited to identify a panel of epidemiologically useful genetic markers. We propose to use a multilocus genotypic analysis of Cryptosporidium isolates from different hosts and from different geographical origins within the New York City watershed.

Methods: A PCR based protocol targeting multiple loci is expected to identify differences between genotypes of isolates from many different potential hosts. DNA is being isolated directly from whole fecal samples by a method modified after Zhu et al. (7), which uses glass beads to break the oocyst wall or by a freeze-thaw method designed in our laboratory.

Samples that have tested positive for Cryptosporidium oocysts at this stage will be further analyzed with a nested multiplex PCR method to verify its genotype (3,4).

In these proposed studies, in addition to expanding our markers to include additional species of Cryptosporidium, we will reexamine the 478 samples that were confirmed by the flotation method. The reexamination will be by PCR only. By doing so, we will be able to see whether all the samples had only one genotype or were contaminated with different species of Cryptosporidium. Furthermore, we will examine an equal number of randomly selected samples from the pool of negatives.